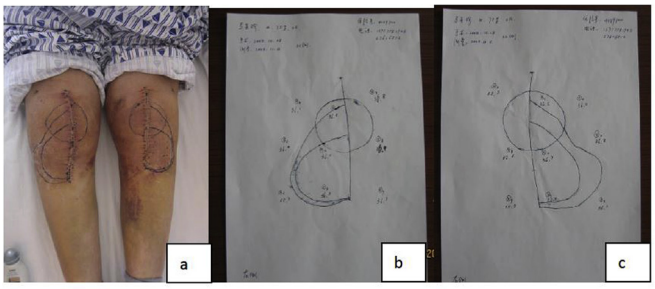
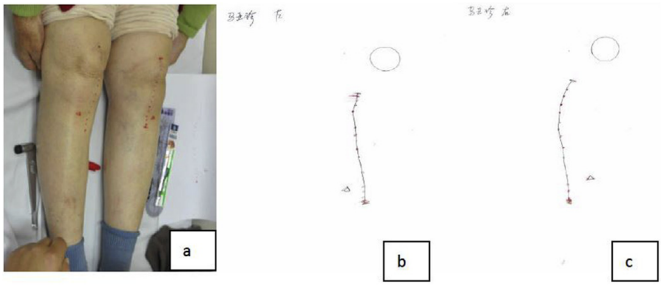


a. Picture of the analgesia and hypalgesia areas measurement at 1 week after TKA.
b. 1:1 pattern of imprinting for left knee.
c. 1:1 pattern of imprinting for right knee.



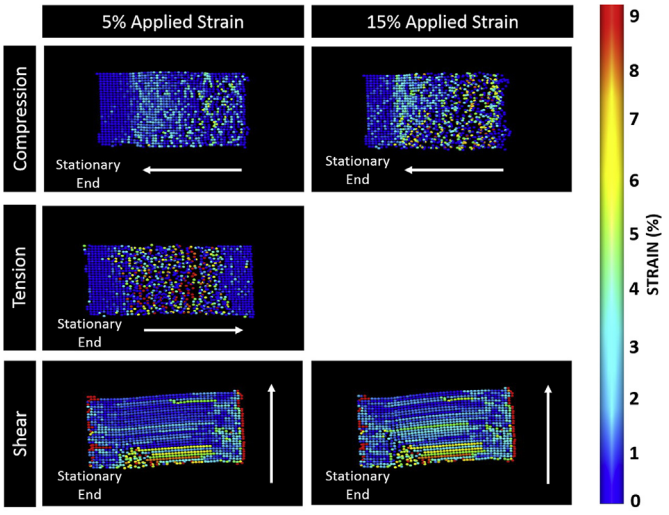
a. Picture of the hypalgesia area measurement at 5 years after TKA.
b. 1:1 pattern of imprinting for left knee.
c. 1:1 pattern of imprinting for right knee.

Tissue Engineering

695
STRAIN DISTRIBUTION AND CELL DEFORMATION WITHIN A 3D HYDROGEL IS INHOMOGENEOUS

S. Leung, S. MacGlashan, D. Musson, J. Cornish, I. Anderson, V. Shim. *Univ. of Auckland, Auckland, New Zealand*

Purpose: Joint loading in vivo results in a complex combination of compressive, tensile, and shear deformations in cartilage. Such mechanical loading plays a crucial role in chondrocytes' development and cartilage health. In vitro models that use 3D hydrogels, such as agarose, have been widely used in studies of how mechanical stimulus influences chondrocyte behaviour. However, due to the inhomogeneity of hydrogels, it is difficult to accurately characterize how mechanical load affects chondrocytes mechanotransduction. Therefore, our objectives were to identify the macroscopic strain distribution within a chondrocyte-hydrogel model (namely surface, middle and bottom regions) under the three modes of loading (compression, tension and shear) and compare it to the local microscopic strain chondrocytes experience over a period of development. This will provide a more accurate understanding on how macroscopic mechanical load affects chondrocytes over their development period.
Methods: Murine chondrocytes seeded in 3% agarose at 4x106 cells/ml were cast between specially designed stainless steel porous-ends to form 15mm×13mm×3mm constructs and was cultured up to 21 days. Constructs were tested on day 1, 3, 7, 14 and 21 time points. Gel-level strain: Cell-gel blocks were placed in a novel device that applied compression, tension and shear at 100μm increments to a maximum of 15% strain in unconfined conditions. Carbon powder was used to trace gel movement. The deformation of the surface, middle and bottom regions of the cell-gel blocks were captured at each increment. Cross-correlation technique was used to calculate strains at each region. Cell-level strain: Cell-gel blocks in the novel device was mounted on an inverted microscope, and the same loading conditions were applied to the cell-gel blocks. The deformation of cells within each region were captured and the deformation index were measured.
Results: The strain distribution within the construct under compression, tension and shear was inhomogeneous across the gel. An example of this is shown in the figure, where it shows the strain distribution at the surface region of the construct under compression, tension and shear loading at day 1 of culture. The internal distribution of strain within the construct, from surface, middle and bottom, was also inhomogeneous. This was also reflected in the deformation of the cells, measured by the aspect ratio. This inhomogeneity means that cells in different regions within a hydrogel construct will receive different amounts of strains under compression, tension and shear loading conditions. The total magnitude of strain that chondrocytes received over the culture period decreased due to the formation of extracellular matrix, which varied within different regions of the construct as well. This may be due to the differences in the nutrient diffusion distance that affects the cell development.
Conclusions: Our results show the strains within chondrocyte-hydrogel constructs vary significantly under compressive, tension and shear loads from region to region. These macroscopic strain inhomogeneities are also propagated down to the local microscopic strain on chondrocytes. Therefore it is important to characterize strain field within the gel and examine cells from different regions separately. If homogenized during bio-analysis, the significant difference in internal strains of the gel is likely to influence the results. Therefore, we believe that it is important to consider location dependent strain inhomogeneity when applying mechanical stimulation using 3D hydrogel systems.



Arrows indicated direction of load

696
AN ENGINEERED BIOMIMETIC CARTILAGINOUS TISSUE MODEL FOR OSTEOARTHRITIS DRUG EVALUATION

Y. Peck, D. Wang. *Nanyang Technological Univ., Singapore, Singapore*

Purpose: Osteoarthritis (OA) is a prevalent degenerative joint disease associated with wear and tear on a joint. Due to an upsurge in the number of potential therapeutic agents, many researchers have heightened their efforts in developing more relevant in vitro cartilage diseased models with the aim of minimizing the use of various animal models for drug screening.
Methods: This study aims to engineer an in vitro 3D cartilage diseased model by co-culturing LPS-activated (LhCG+Mϕ++) or non-activated macrophages (LhCG+Mϕ–) with/in living hyaline cartilage grafts that are made from injectable and removable hydrogel template constructs. The samples were harvested after a co-culture period of 7-days and 14-days, respectively. Subsequently, viability staining, transcript levels, histology and immunohistochemistry were used to assess chondrocyte responses in terms of cell viability and changes in the cartilage matrix composition. Furthermore, chondrocyte hypertrophy and apoptosis were assessed via collagen type X expression and Annexin V/PI staining.
Results: The results showed that cell viability and cell density were significantly higher in the LhCG+Mϕ++as compared to the LhCG+Mϕ–